tents was unreasonably long (about 8 min.) in contrast to the time (1-2 min.) required for the destruction of gossypol at 200°C. and above.

In order to circumvent the prolonged heat-up interval, use was made of a sample container that at first glance seems an unlikely high temperature-pressure device, namely a sealed glass vial. Heat-up intervals of less than 60 seconds were achieved, using the glass vials. The vials were prepared by sealing one end of a 2-in. length of 6-ml. Pyrex tube. Approximately ½ g. of soapstock was then introduced into the tube with a large bore hypodermic needle and syringe. This technique allows the vial to be filled easily and prevents smearing the upper walls of the tube at the point where the final seal will be made. For treatment the sealed vials, with about one-quarter of the total volume left as free head space above the soapstock sample, were immersed in an oil bath preheated to the requisite temperature. Dozens of the sealed vial experiments were carried out at temperatures up to 240°C. without a single failure from either thermal shock or internally developed pressure.

Table IV shows data obtained by the sealed glass vial technique for the heat treatment of raw, alkaline soapstock. Table V shows similar data for acidulated soapstock. Alkaline soapstock is far easier to free from gossypol by simple heat treatment than is acidulated soapstock.

TABLE IV

Effect of Heating Alkaline Soapstock at Temperatures Above 100°C.

| | % Gossypol | |
|---|---|---|
| | Free | Total |
| Control | 3.76 | 4.20 |
| 180°C. (4 minutes) | 0.54 | 0.67 |
| 190°C. (4 minutes) | 0.15 | 0.16 |
| 200°C. (4 minutes) | 0 | 0 |
| 210°C. (2 minutes) | 0 | 0 |
| 220°C. (1 minute) | 0.30 | 0.44 |
| 220°C. (2 minutes) | 0 | 0 |
| 240°C. (1 minute) | 0.15 | 0.32 |

TABLE V

Effect of Heating Acidulated Soapstock at Temperatures Above 100°C.

| | % Gossypol | |
|---|---|---|
| | Free | Total |
| Control | 6.2 | 6.2 |
| 180°C. (4 minutes) | 5.6 | 5.4 |
| 180°C. (8 minutes) | 4.5 | 4.9 |
| 190°C. (4 minutes) | 5.0 | 5.1 |
| 190°C. (8 minutes) | 3.8 | 4.1 |
| 210°C. (1 minute) | 5.8 | 5.7 |
| 210°C. (2 minutes) | 4.6 | 5.1 |
| 210°C. (4 minutes) | 3.6 | 4.0 |

Since presently there seems to exist no adequate substitute for feeding tests insofar as the evaluation of gossypol toxicity is concerned, a pilot plant scale heat heating device is being constructed for the preparation of amounts of material sufficient for this purpose.

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# Castor Seed Proteins and Their Viscosities

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INDIA produces oilseeds to the tune of 675,000 tons *per annum*, out of which the production of castor seeds accounts for 118,000 tons according to the average figure up to 1950. Except for some quantity of castor seeds which is exported, castor seeds are hydraulically pressed to remove oil, and the cake is partly exported and partly used as a fertilizer, especially for sugar cane crops. The castor seed meal is usually first expressed cold and then hot at higher pressures. The proteins of the cake get partly denatured during the course of hot-pressing. In this paper an attempt has been made to obtain white, high-grade, undenatured proteins from seed kernels prior to extraction of oil by the usual methods of pressing and solvent-extraction. Thus by the adoption of this process both good quality oil and undenatured, high-grade proteins will be obtained, which can be used as casein substitute.

Studies on the castor proteins date back to Ritthausen's period (1) when systematic work was carried out for the first time in 1857 on the isolation and nature of proteins from castor seeds, which was confirmed and largely supplemented by the more exhaustive work of Osborne and his associates in the Connecticut Agricultural Experiment Station (2).

Ritthausen's dialysis of the extract of deoiled castor cake with 10% sodium chloride yielded crystalline globulin 12.4%, composed of $\alpha$-globulin coagulating at 86°C. and $\beta$-globulin coagulating at 96°C. The globulin thus obtained gave an analysis, arginine 13.19%, histidine 2.74%, and lysine 1.54%. In 1888 Stillmark (3) showed that castor meal also contained a toxalbumin called ricin to the extent of 1.5%. Later work by Spies and collaborators (4) have shown that the castor cake contains an allergic protein polysaccharidic fraction or "pentose" protein to the extent of 1.8%, which causes asthma on prolonged inhalation.

The work on vegetable proteins by different workers has indicated that the proteins of various oilseeds consist mainly of glutelins, $\alpha$-globulins, and $\beta$-globulins; and there is a similarity of behavior on the part of proteins from various oilseeds although there could be differences in their properties. The proteins (1, 2) obtained from castor meal have been found to be almost similar in properties to soybean and peanut. Investigations on the peptization of castor seed proteins

have been carried out and their use has been indicated in the preparation of adhesives (5), as binders in water paints and distempers (6), plastics and fibers (1), leather finishes, paper sizing, glutamic acid (7), monosodium glutamate (8), and several other industries.

Kamath and Kulkarni (9) have evaluated the properties of castor proteins from defatted, pressed cake and studied their usefulness in distempers, oil-bound water paints, adhesive compositions and plastic products. However the fibers made from these proteins were brittle and had a brownish shade. White proteins are reported (9) to have been prepared from coarsely ground castor beans by pretreatment with 10% NaCl at 50°C., followed by filtration and acidification. In the present work attempt has been made to recover white, high-grade, undenatured proteins from castor bean kernels instead of castor beans, using 10% common salt as the peptizing agent and $SO_2$ as the precipitating agent, 0.2% caustic soda as the peptizing agent, and acetic acid and $SO_2$ as precipitating agents and to evaluate their properties as to ash content, nitrogen content, and viscosities. The last have been compared with acid casein. It was also considered that the residual meal left after protein extraction would be likely to give a higher yield of oil on pressing than was obtainable by the conventional methods of pressing. The liquor left after the recovery of proteins may be worked out for the recovery of carbohydrates and inositol hexaphosphate (11). The latter may be hydrolyzed to yield inositol, which has great value in the pharmaceutical industry as a member of the vitamin B complex, in multi-vitamin preparations, for treatment of disturbances in fat and cholestrol metabolism and of diabetes mellitus. Large-scale experiments are being arranged for the recovery of oil from seed meal left after the recovery of proteins.

## Experimental

Cleaned castor seeds were decorticated and reduced to coarse powder (5 mesh). Then 1 kg. of coarsely crushed castor bean kernels were soaked in 10 liters of 10% NaCl solution at 50–60°C. for 2 hrs. The seed meal was allowed to settle, and the supernatant saline solution was decanted off. The lower layer containing the slurry mass of the seed meal was filtered and then washed with a small amount of water. The washings were mixed with the decanted solution, from which proteins were precipitated with 5% acetic acid at pH 3.0. The solution was diluted with water by three times the original volume when the precipitate was obtained in a granular form which could be easily filtered. The protein curd obtained on the filter paper was washed several times with distilled water and then dried 4 times with 98% alcohol and 4 times with dry acetone as it was found that proteins on drying at atmospheric or reduced pressure at room temperature turned to a horny, translucent mass. This showed a marked decrease in swelling in water and in the solubility of the usual protein solvents.

The final acetone-washed sample was kept under reduced pressure at room temperature, evaporating the acetone and leaving the proteins in the form of white powder, which had the usual property of swelling in water and dissolving in salt or alkaline solutions. The percentage yield and ash and nitrogen content of the samples were determined. Similar experiments were repeated, using 10% NaCl as peptizing agent and $SO_2$ as precipitating agent, 0.2% NaOH as peptizing agent, and acetic acid and $SO_2$ as precipitating agents. The results as to yield and other properties are given in Table I.

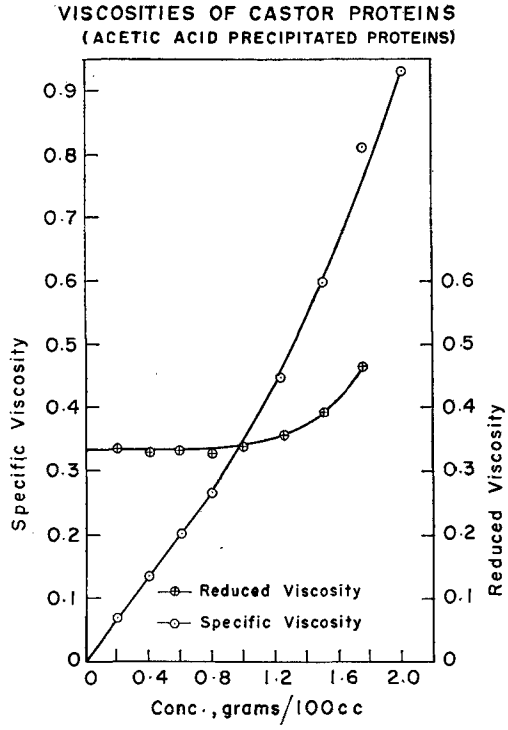


FIG. 1

## Viscosity Measurements

First, 0.5 g. of the protein sample was weighed in a 100-cc. beaker covered with watch glass, then soaked in 2% of double distilled water and kept for 18-20 hours. After the requisite soaking in water the proteins were dissolved in 23 cc. of 0.2% NaOH solution,

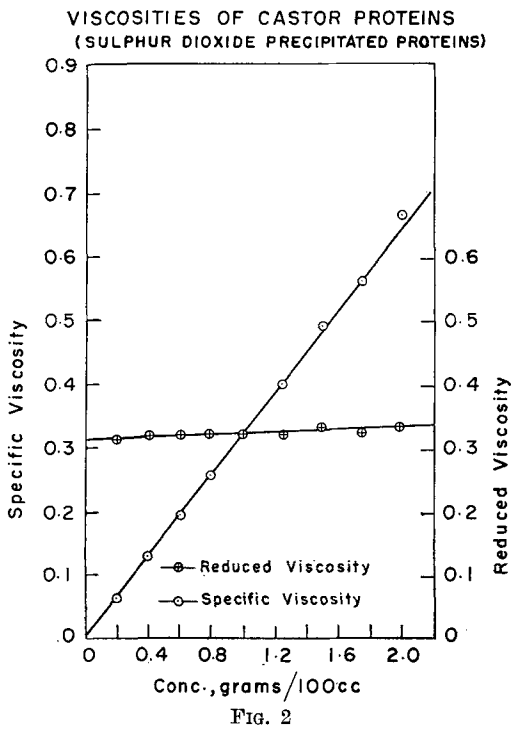


FIG. 2

TABLE I
Properties of Castor Proteins

| Sample | Peptizing agent | Precipitating agent | Yield, % | Ash content | Nitrogen content, % | Color |
|---|---|---|---|---|---|---|
| I | NaCl 10% | Alcohol | 6.5 | 0.681 | 15.2 | White |
| II | NaCl 10% | Acetic acid | 5.4 | 0.561 | 16.0 | White |
| III | NaCl 10% | HCl 5% | 3.5 | 0.612 | 15.9 | White |
| IV | NaCl 10% | $SO_2$ | 5.8 | 0.482 | 15.1 | Pale yellow |
| V | NaOH 0.2% | Alcohol | 9.6 | 1.98 | 15.7 | White |
| VI | NaOH 0.2% | Acetic acid | 8.5 | 1.79 | 15.9 | White |
| VII | NaOH 0.2% | HCl 5% | 6.5 | 1.85 | 15.8 | White |
| VIII | NaOH 0.2% | $SO_2$ | 9.1 | 1.35 | 14.8 | Pale yellow |

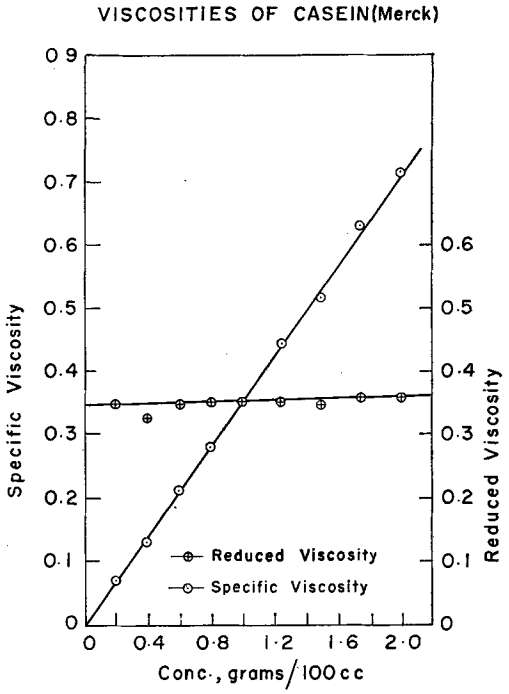


FIG. 3

which earlier was made carbonate-free by precipitating the carbonate with the addition of a calculated amount of barium hydroxide, decanting and filtering through glass wool, and preserving it in an aspirator bottle with a soda lime guard tube and tapping arrangement. The solubilization of proteins was effected by warming the contents for 10 min. on a water bath at 60°C. and filtering through glass wool.

Viscosities were determined by employing an Ostwald U-tube viscometer in a thermostat at 25 ± 1°C. The 2.0% solution of proteins was diluted with double distilled water to various lower concentrations, and the time of flow was observed in each case. The mean of four readings was taken as final. It was observed that dilution with 0.2% alkali solution in place of water did not make any appreciable difference in the values of specific viscosities obtained. The relative, specific, and reduced viscosities were determined at various concentrations for castor proteins, peptized with 0.2% NaOH and precipitated with acetic acid and $SO_2$ and acid casein (Merck), each separately. The graphs of specific and reduced viscosity against concentrations for the three types of proteins are shown in Figures 1, 2, and 3.

From Table I it is clear that the properties of proteins obtained from castor bean kernels, using different peptizing and precipitating agents, are almost similar. The percentage yields are highest in Experiments V, VI, and VIII. The quantity of castor proteins obviously will be better in Experiments I, II, III, and IV as proteins peptized with 10% NaCl do not contain any fibrous matter and are thus better adapted, especially for making fibers. However for various industrial purposes or as a substitute for acid casein it would be economical to use 0.2% NaOH as peptizing agent, employing acetic acid or $SO_2$ as precipitating agent. Moreover, as sulphuric acid and hydrochloric acid had a denaturing effect on the proteins, it is preferable to use weakly ionizing acids like acetic acid and sulphurous acid as precipitating agents. For these reasons protein samples peptized with 0.2% NaOH solution and precipitated with acetic acid or $SO_2$ were chosen for the comparative study of viscosities with respect to acid casein of Merck quality.

By referring to Figures 1, 2, and 3 it can be seen that at 1% concentration, intrinsic viscosities of NaOH peptized acetic acid precipitated proteins is 0.332 and of NaOH peptized —$SO_2$ precipitated proteins is 0.314, while that of acid casein of Merck quality is 0.345. These results definitely show that if castor bean kernels are processed for the recovery of proteins prior to oil extraction, good quality proteins may be made available having properties comparable to acid casein. They can be employed as casein substitute for various industrial purposes.

## Summary

Properties of castor-proteins from castor bean kernels prior to extraction of oil have been studied with regard to ash content, nitrogen content, and yields, using different peptizing and precipitating agents. The viscosities of the NaOH peptized and acetic acid or $SO_2$ precipitated proteins were determined and found to be comparable to acid casein. Maximum yields were obtained by using alkali as a peptizing agent and acetic acid or $SO_2$ as precipitating agents. These castor proteins may possibly be adopted as a substitute for casein in various industries.

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